

INFO-RESEARCH...

Friedreich's Ataxia: New developments and perspectives

By Dr. Massimo Pandolfo

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I will try to present summarily where we are in our research in understanding the causes of Friedreich's ataxia (FA) and to give you a general idea of the direction the research is taking and that which we foresee discovering in the next few years.

It was in 1863 that Professor Nicolas Friedreich, a professor in medicine in Heidelberg in Germany, described the disease. Researchers and clinicians had a detailed description of the clinical picture of this disease, but they did not know its cause. All that we knew was that the disease was hereditary, according to a recessive mode of heredity. We had no idea of what was the gene, which protein it contained the information of, and for what reason the afflicted people developed symptoms. In 1996 we finally identified the gene of FA, that is to say the segment of DNA containing the genetic information which is abnormal in the people who have the disease.

The gene is situated on chromosome 9 and contains the information necessary for the building of a protein called frataxine. The function of frataxine was not able to be determined at the time when the gene was cloned. The first discovery, after the discovery of the gene, was the understanding of the abnormality on the level of the DNA. We found that the majority of individuals who have FA are carriers of an unstable expansion of a repetition of a trinucleotide, that is, an excessive number of repetitions of a DNA sequence composed of three unities (GAA). The GAA trinucleotide, instead of being repeated less than 40 times, as is the case on a normal chromosome, is repeated between 100 and 700 times and sometimes more than 1,000 times. Since then, we have learned that an excessive number of repetitions do not cause the production of an abnormal protein, as is the case in all dominant ataxias, but cause rather a deficiency in a normal protein. The fact of having too many repetitions causes the patient afflicted with FA to build in a small quantity a protein which he needs in a much greater quantity. The size and the sequence of amino acids of frataxine are perfectly normal in the patient, there are simply too few. It is the consequence of the expansion.

During the 3 years which followed the discovery of the gene, we also realized that this expansion of a trinucleotide explained at least in part the clinical variability observed in FA. The age at its beginning, the speed of its progress, and its severity and the area of neurons most severely afflicted. These are the same sensitive neurons responsible for the sense of positioning which is greatly impaired in this disease. The heart, which normally builds greater quantities of frataxine than the other organs of the body, is afflicted by the disease. We should remember this when we try to understand the consequences of a deficit in frataxine. The cells build different quantities of frataxine. They are therefore not equally sensitive to a deficit in frataxine.

The other important discovery was to locate frataxine in the cellular structure, that is to say, in which structure in the interior of the cell frataxine is found. We and other researchers carried out several experiments which all confirm that frataxine is built in the mitochondria. What are the mitochondria? They are thousands of small structures in the interior of every cell of our body. They are the generators of energy of the cell. The mitochondria are the structures of the cell where a very important chemical reaction is produced called cellular respiration. It is the place where the chemical compounds coming from the food which we eat are burned to generate energy. The molecules which come from $\text{C}_6\text{H}_{12}\text{O}_6$ and oxygen are combined to produce energy in the mitochondria. Frataxine is localized in the structure which generates energy and which burns food in order to produce energy.

What is the function of frataxine in the interior of the mitochondria? The elucidation of this problem is a great challenge. It does not resemble any of the proteins of which we know the function. However, the fact that we have realized that all living organisms build a protein which resembles frataxine helps us: mice, fruit flies, worms and baker's yeast. Baker's yeast builds a protein which is almost identical to human frataxine and is localized in the mitochondria of the yeast. The fact that yeast builds a protein identical to frataxine is a great advantage because it is easier to do genetic manipulations in yeast than in mice.

Some researchers, such as Dr. Jerry Kaplan of the University of Utah, produced cells of yeast which do not contain frataxine. The most striking thing concerning these cells which do not have frataxine is the severe disruption of the metabolism of iron. These yeast cells incorporate much more iron than normal yeast cells and the excess of the iron incorporated by the cells are found in the mitochondria. The consequence of an excess of iron in the mitochondria make the cells very sensitive to oxidation stress.

In the mitochondria, oxygen circulates through proteinic complexes called complexes of the respiratory chain. A small quantity of oxygen which circulates through these complexes in the mitochondrial membranes can form what we call free radicals. This can bring about the formation of H_2O_2 in the mitochondria and H_2O_2 reacts with iron

to form the hydroxyle radical. We know that the hydroxyle radical is a substance which damages the mitochondrial proteins, the membranes and the DNA. The fact of having too much iron in the mitochondria is not desirable. After having done this initial study in yeast, it was extremely important to see if we could find something in the disease in the human which indicated that the situation was the same. Current data show that the situation is probably the same in the disease in a human. If we do a colouration on the iron on the tissues of the heart coming from patients afflicted with FA, we find deposits of iron. This observation was made around 20 years ago by a Canadian neuropathologist. However, at that time, we were not able to interpret this observation. In the tissues afflicted like the heart, we observe deposits of iron like those which are present in the model of the yeast. These deposits of iron are found in the heart of patients afflicted with FA, but not in the heart of patients who have any other type of heart disease. We observe an abnormal distribution of iron, too much iron in the mitochondria and probably not enough iron outside the mitochondria, rather than a generalized accumulation.

In the 1960's a study was done by a Hungarian neurologist in which he injected radioactive iron into patients afflicted with different neurological diseases. He perceived that the iron was retained in a greater quantity in the brain of patients afflicted with FA than in that of patients afflicted with other diseases or normal individuals. It is another indication buried in medical literature. There is something abnormal in the metabolism of iron in patients afflicted with FA. The tissues which are not affected like the skeletal muscles do not present an accumulation of iron in this disease.

This signifies that it concerns a specific process affecting the tissues. Does iron really accumulate in the mitochondria? We have very exciting recent data which demonstrates that, in the heart of patients afflicted with FA, there is an accumulation of a material which has all of the characteristics of iron in the mitochondria, but we do not see this in the mitochondria of the heart of other persons who have any other cardiac disease. It is very specific to FA. We have started to gather observations according to which the accumulation of iron in the mitochondria are found in the tissues affected by the disease in patients suffering from FA. We are now expanding this study to samples of nervous tissues.

A slight accumulation of mitochondrial iron can be observed in the cells which are not afflicted by the disease like the cells of the skin. What are the consequences of an excess of iron in the mitochondria? The specific consequence is that some mitochondrial enzymes which have a centre containing iron sulphide are inactivated by an excess of iron. This brings about a dysfunction of the respiratory chain and a loss of energy on the level of the cells. Furthermore, this causes a hypersensitivity of the cells to oxidizing agents and the cells which are affected die. We think that the deficiency in frataxine provokes an increase in mitochondrial iron and this provokes an increase in the production of free radicals and damage to the mitochondria. On

one hand, this provokes a lack of energy and the cells do not have enough energy to function adequately. They can provoke the death of nervous fibres as much in the peripheral nerves as in the spinal column. On the other hand, the damage to the mitochondria can directly set off a process called programmed cellular death or cellular suicide.

This might be the mechanism underlying the loss of cells which we observe in the heart and in certain parts of the central nervous system; it is important to say that we have data on this process in cells in laboratory cultures, but we do not have evidence that this process is the cause in the patients. It is a goal of research in the future.

We would like to have an animal model of FA and there are now 7 or 8 different approaches which are being tried out in order to generate an FA mouse model. We hope that in a few months to a year we will have one.

Secondly, the idea that the patients have too much iron in their mitochondria suggests possible treatments to try in order to slow down the progression of the disease. It will be difficult to withdraw the excess of iron because the iron is sequestered in structures where the chelating agents which are now available cannot penetrate efficiently. We can try what we call antioxidants to stop up a part of the free radicals which are produced in this disease. Among the antioxidants used in the laboratory, derivatives of co-enzyme Q seem to be the most efficient in limiting the toxicity of iron for mitochondrial structures. A few research groups like the French group are attempting to evaluate this substance by a controlled therapeutic trial in patients and we plan to do a similar study in Montreal. We expect a slowing down in the progression of the disease as suggested by the preliminary data. The effect may be limited and transitory. Therefore we should not put too much hope in it. What is important is that even if the first treatment which we try does not resolve the problem, we will begin to understand the process of the disease, which will allow us in the future to identify possible targets for therapeutic trials in patients. This constitutes a great revolution since the time when the gene was cloned.

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